REVIEWED
By Anne Nord at 1:15 pm, Apr 30, 2021

4/29/2021

Worklist: 4939

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2021-0871	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0876	6	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0879	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0888	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0931	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0974	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0975	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 04/29/21 Analyst: Britany Wylie
Plate lot#: ~~201412~~ 210412 BW Plate Expiration: ~~6-6-2021~~ 10-12-21 BW
Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE LCMS Methanol Hexane
Blank Blood Lot: 20K20702 **Column**: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 69679

Pre-Analytic:

☒ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
☒ 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
☒ 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette **1000µL blood (calibrated pipette) Pipette ID: k52558g** in wells of analytical (standards) plate.
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 4. Pipette **500µL 0.1% formic acid in water blood sample** in wells of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **800µL of blood+acid or urine acid** mixture to corresponding wells of SLE+ plate.
☒ 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right) Manifold ID: 66792
☒ 8. Wait 5 minutes.
☒ 9. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
☒ 10. Wait 5 minutes.
☒ 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 12. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
☒ 13. Wait 5 minutes.
☒ 14. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
SPE Dry ID: 66819
☒ 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

☒ 1. Create batch and process data.
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥0.98 for each analyte
☒ 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less).
Ion ratios must be within +/- 20% of the averaged calibrators
☒ 4. Case sample response for THC and OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
☒ 5. Did all QCs pass for each analyte? (if not is it describe in comments section)
☒ 6 Enter QCs into control charting.
☒ 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Blood only batch; curves limitations: THC-OH 3-100

am 27
4/29/21 worklist 4939

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood				IS + QC_1
B	IS + Cal. 2	871-1				IS + Cal. 7
C	IS + Cal. 3	876-6				IS + Cal. 6
D	IS + Cal. 4	879-1				IS + Cal. 5
E	IS + Cal. 5	888-1				IS + Cal. 4
F	IS + Cal. 6	931-1				IS + Cal. 3
G	IS + Cal. 7	974-1				IS + Cal. 2
H	IS + QC_1	975-1				IS + Cal. 1

All wells to contain 100 µl of residual DMSO

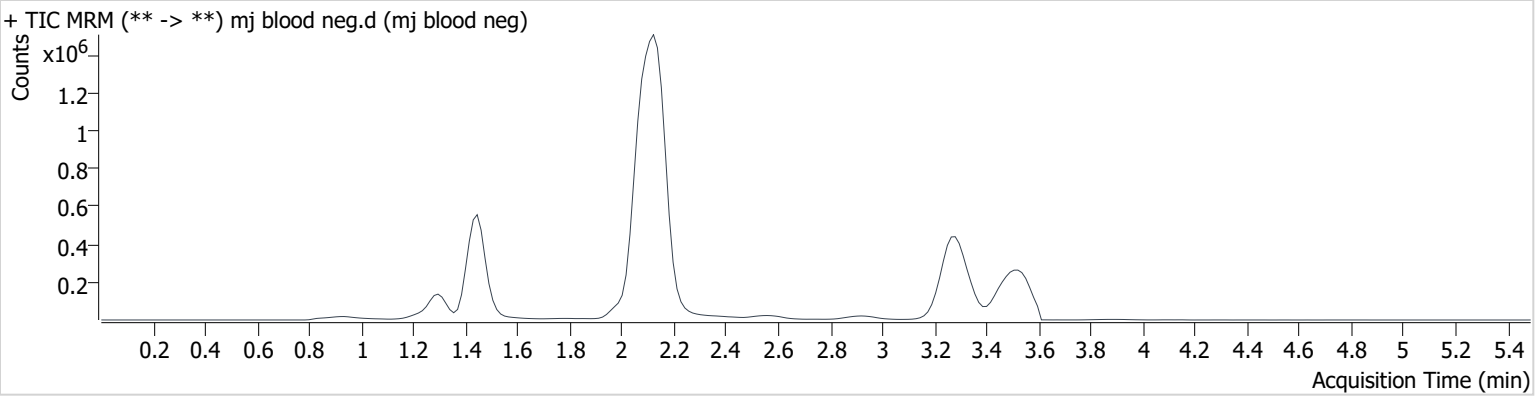
Case #: _C2021-0____-1

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj blood neg.d
Type	Sample	**Sample**	mj blood neg
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:36:11 PM		
Sample Info.			

Sample Chromatogram

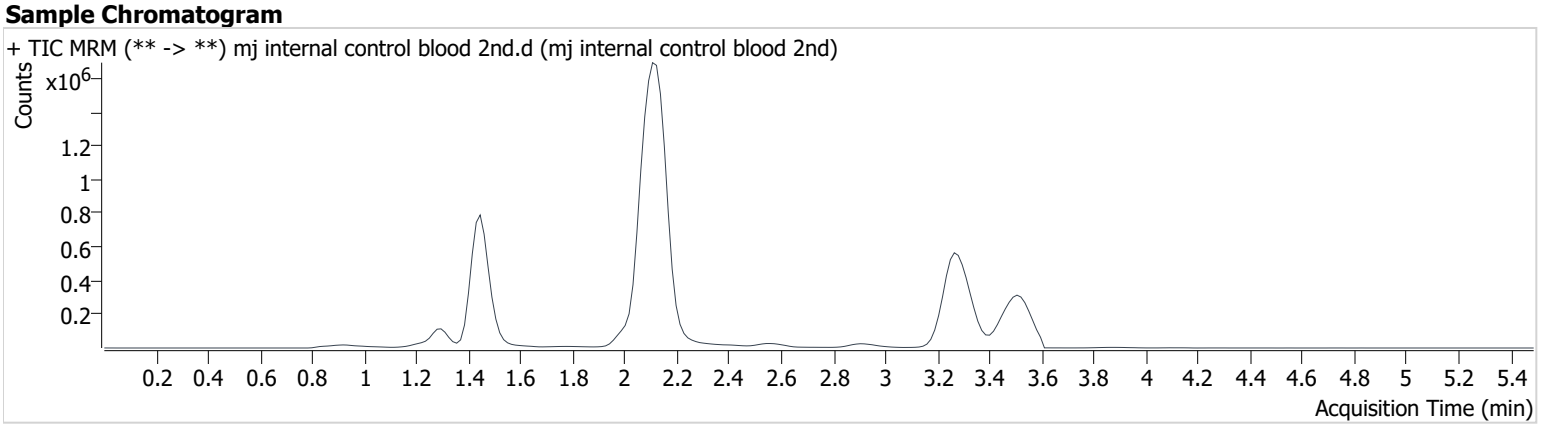


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj internal control blood 2nd.d
Type	QC	**Sample**	mj internal control blood 2nd
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 9:16:17 PM		
Sample Info.			

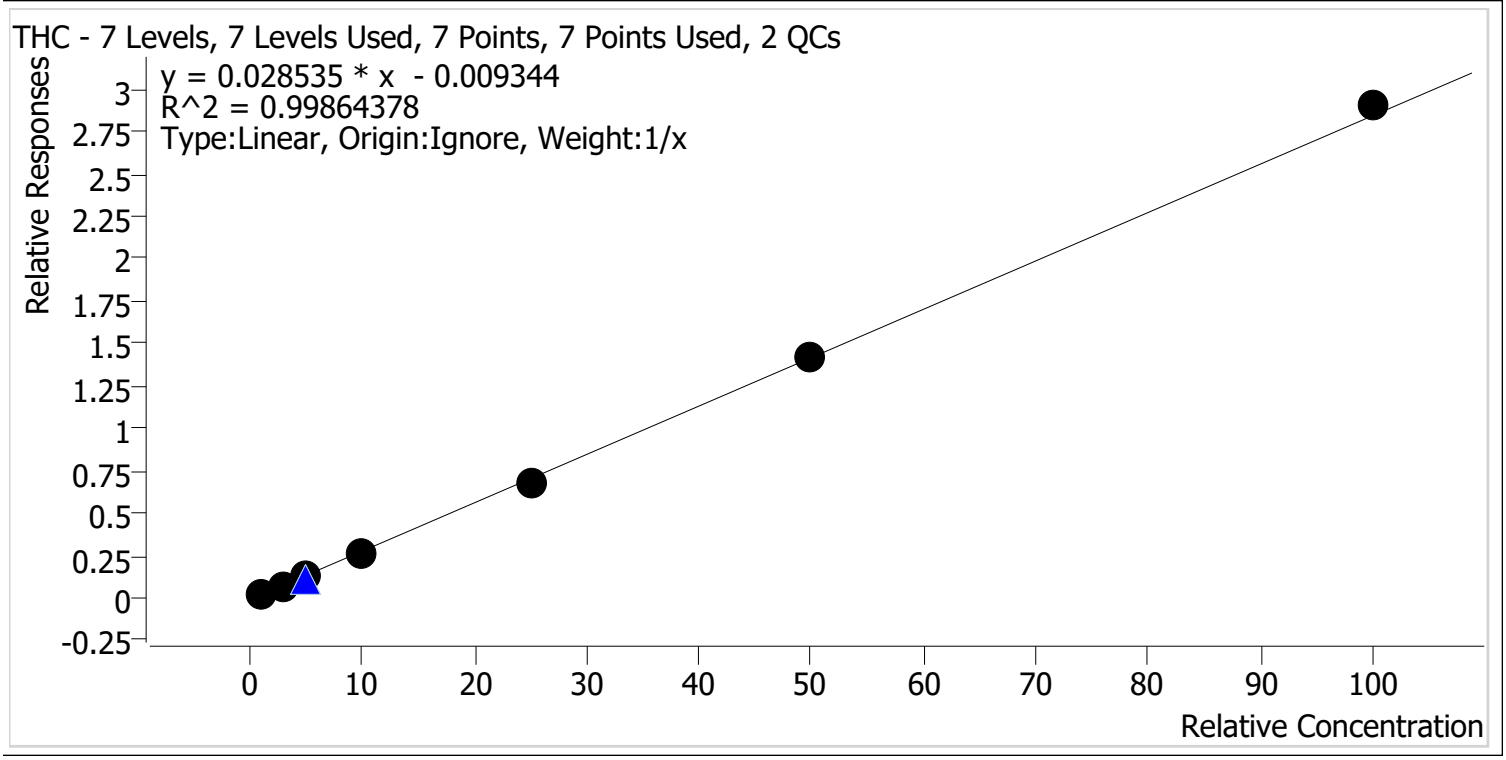
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	317601	∞	11.0	∞	2275368	4.718	ng/ml
THC-COOH	1.476	163025	305.9	35.8	859.9	837437	14.308	ng/ml
THC	3.317	183243	∞	24.3	∞	1618508	4.295	ng/ml

Compound Calibration Report

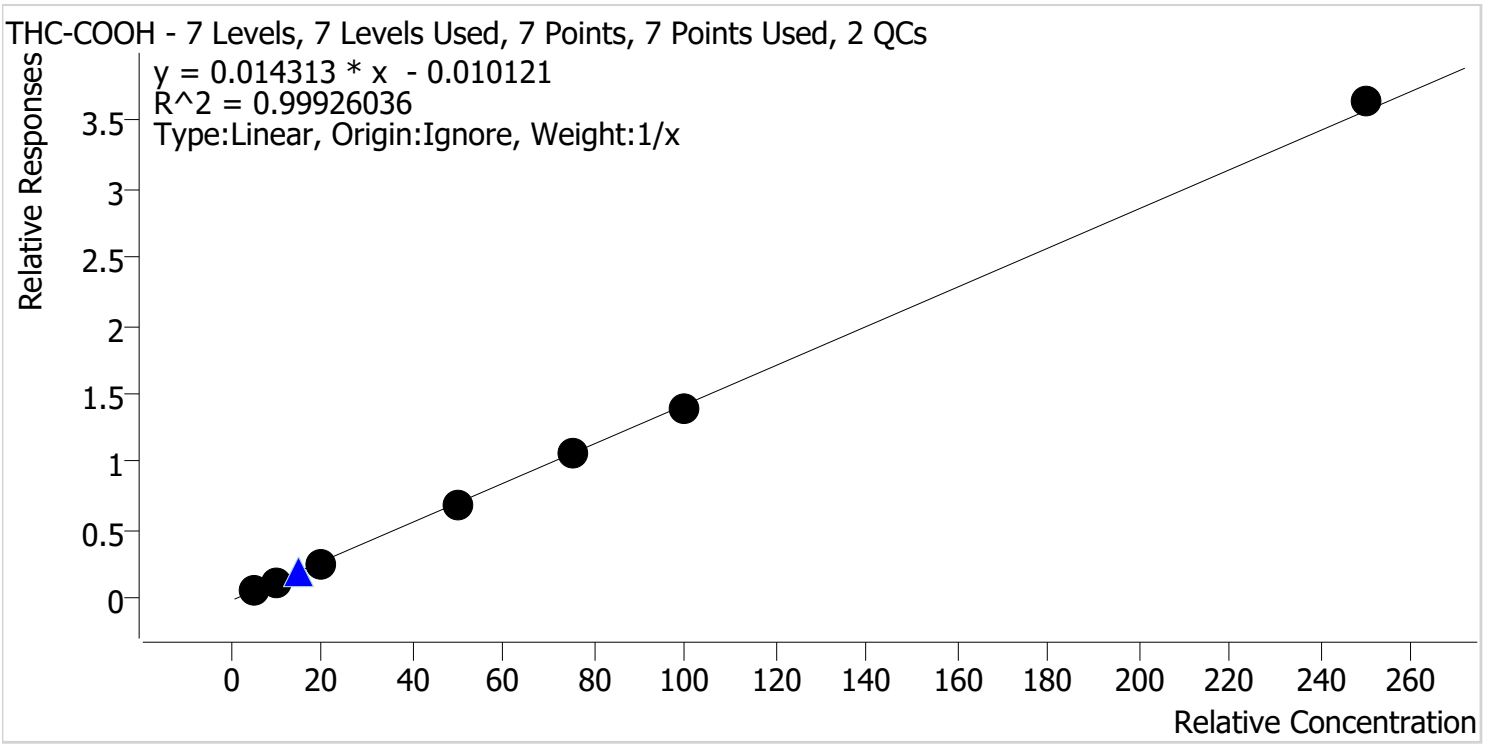
Batch results	D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Last Cal. Update	4/29/2021 9:26 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	121.9
mj cal2	2	✔	3.0	2.8	94.7
mj cal 3	3	✔	5.0	4.6	92.8
mj cal 4	4	✔	10.0	9.3	92.8
mj cal 5	5	✔	25.0	23.9	95.6
mj cal 6	6	✔	50.0	50.1	100.1
mj cal 7	7	✔	100.0	102.0	102.0

Compound Calibration Report

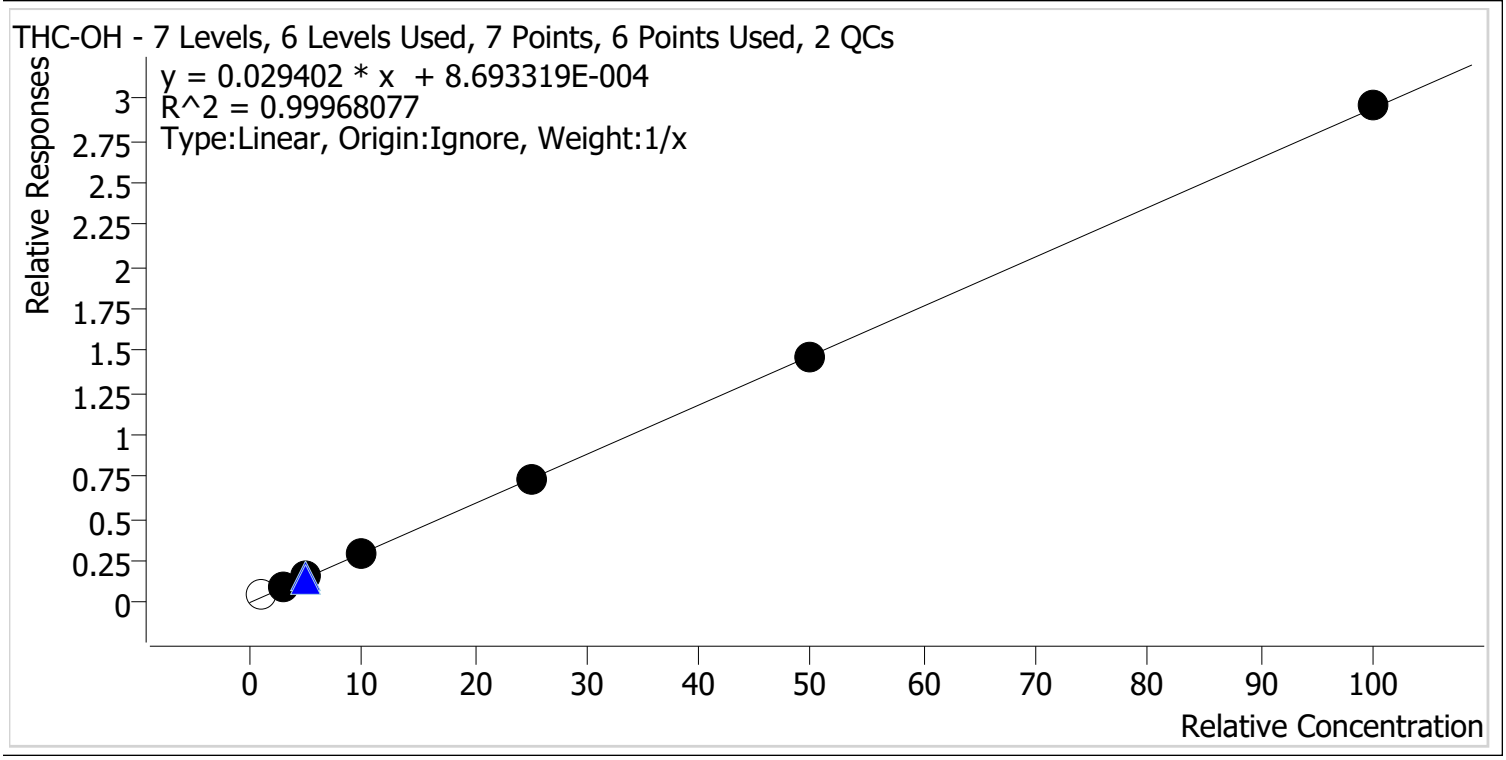
Batch results	D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Last Cal. Update	4/29/2021 9:26 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	5.0	5.5	110.6
mj cal2	2	✔	10.0	9.8	97.9
mj cal 3	3	✔	20.0	19.1	95.4
mj cal 4	4	✔	50.0	48.4	96.9
mj cal 5	5	✔	75.0	74.6	99.4
mj cal 6	6	✔	100.0	97.9	97.9
mj cal 7	7	✔	250.0	254.7	101.9

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Last Cal. Update	4/29/2021 9:26 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



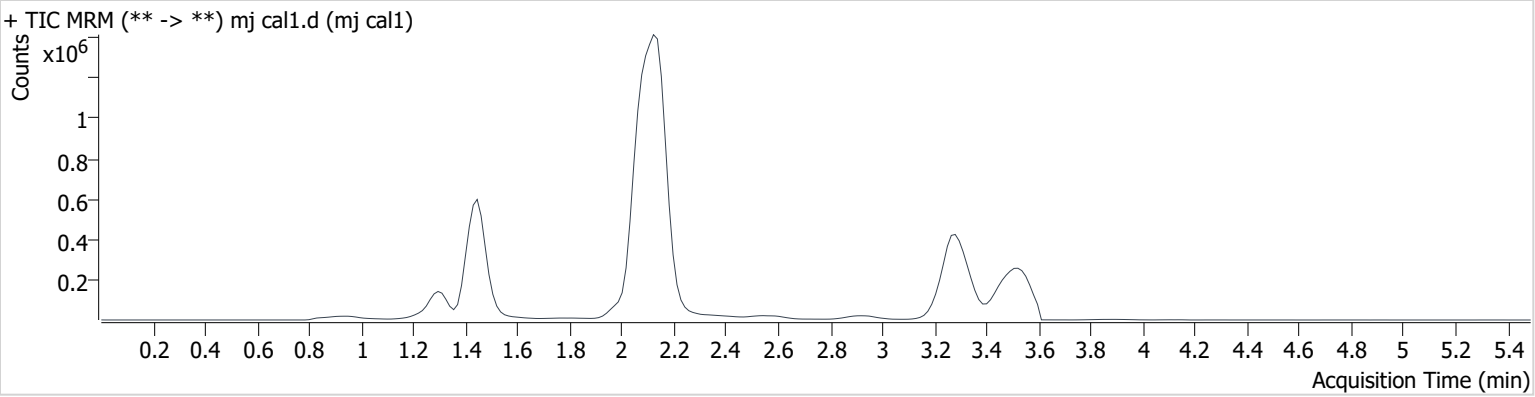
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✗	1.0	1.8	179.1
mj cal2	2	✔	3.0	2.8	94.1
mj cal 3	3	✔	5.0	5.3	106.4
mj cal 4	4	✔	10.0	10.1	101.3
mj cal 5	5	✔	25.0	24.7	98.6
mj cal 6	6	✔	50.0	49.5	99.0
mj cal 7	7	✔	100.0	100.6	100.6

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal1.d
Type	Cal	**Sample**	mj cal1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 6:42:30 PM		
Sample Info.			

Sample Chromatogram



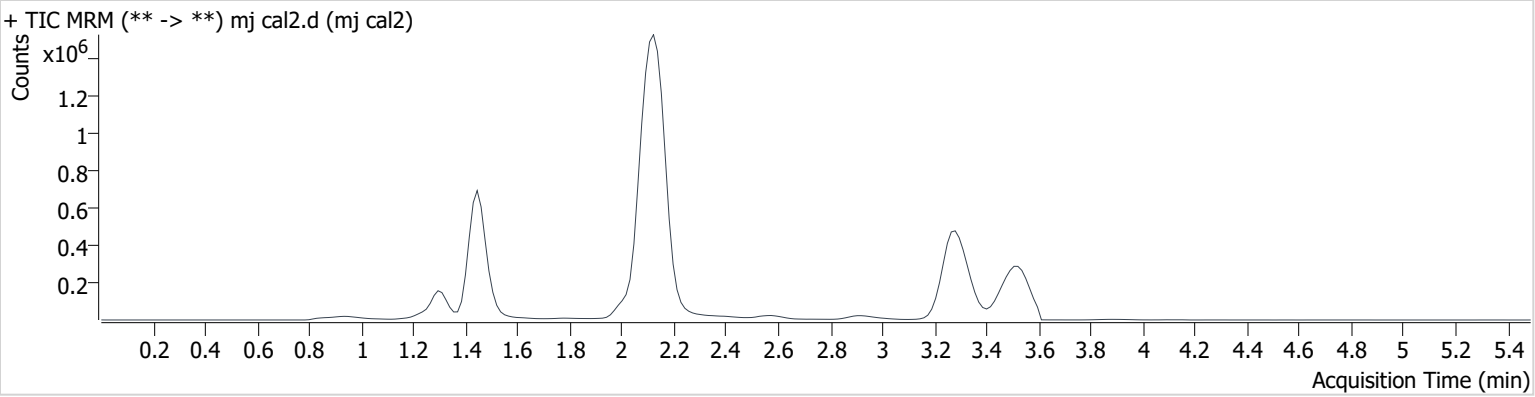
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	118549	∞	5.9 **Low**	∞	2214747	1.791	ng/ml **Low**
THC-COOH	1.476	51523	89630.9	33.1	81.5	746272	5.531	ng/ml
THC	3.317	31606	∞	25.7	1303986460438.0	1242743	1.219	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal2.d
Type	Cal	**Sample**	mj cal2
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 6:49:14 PM		
Sample Info.			

Sample Chromatogram



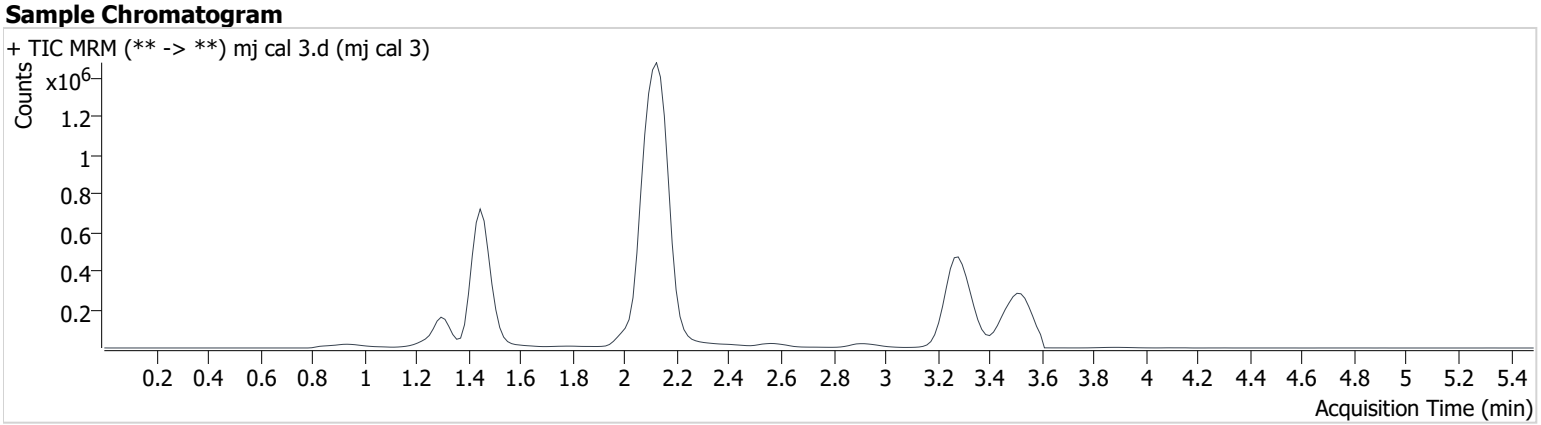
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	177345	∞	10.4	∞	2114097	2.824	ng/ml Low
THC-COOH	1.476	94701	521.3	32.0	27.2	728352	9.791	ng/ml
THC	3.317	91004	∞	25.7	4157842 3955399 .3	1268562	2.841	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 6:55:57 PM		
Sample Info.			

Sample Chromatogram



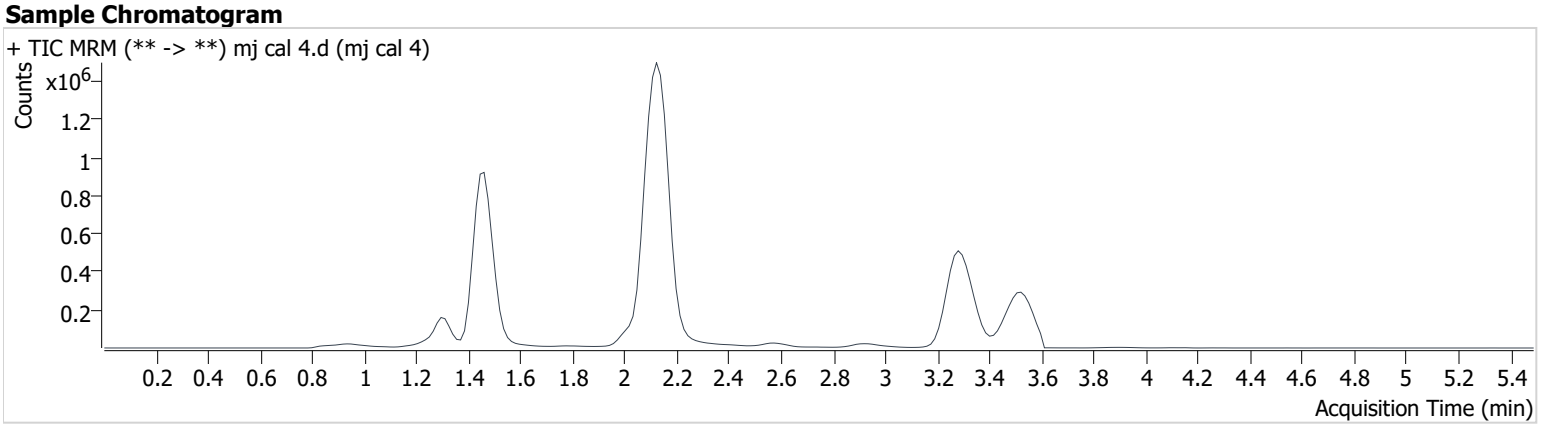
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	329270	∞	9.6	∞	2093443	5.320	ng/ml
THC-COOH	1.476	192858	2147.5	35.7	121.4	733330	19.081	ng/ml
THC	3.317	150991	∞	25.1	∞	1226441	4.642	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:02:39 PM		
Sample Info.			

Sample Chromatogram



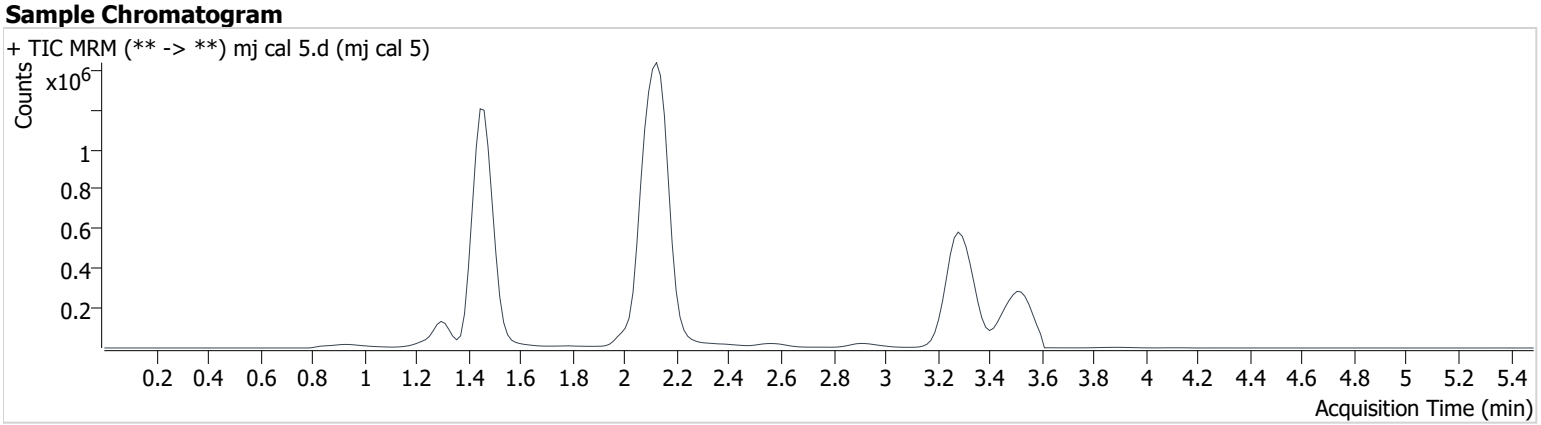
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	615562	∞	10.4	∞	2061080	10.128	ng/ml
THC-COOH	1.476	495975	1091.9	35.9	393446.6	726099	48.430	ng/ml
THC	3.332	308170	∞	24.9	40790870874596.9	1206570	9.278	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:09:21 PM		
Sample Info.			

Sample Chromatogram



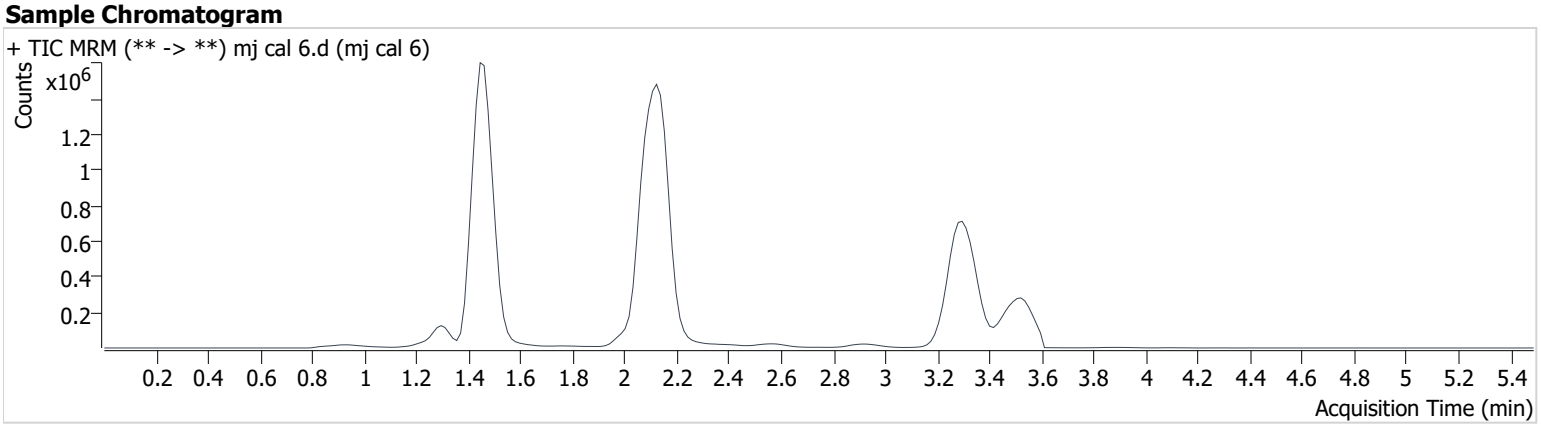
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	1507312	∞	11.1	∞	2076830	24.655	ng/ml
THC-COOH	1.476	760398	1245184.3	36.0	782.7	719322	74.563	ng/ml
THC	3.317	859702	∞	23.8	∞	1278060	23.901	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:16:03 PM		
Sample Info.			

Sample Chromatogram



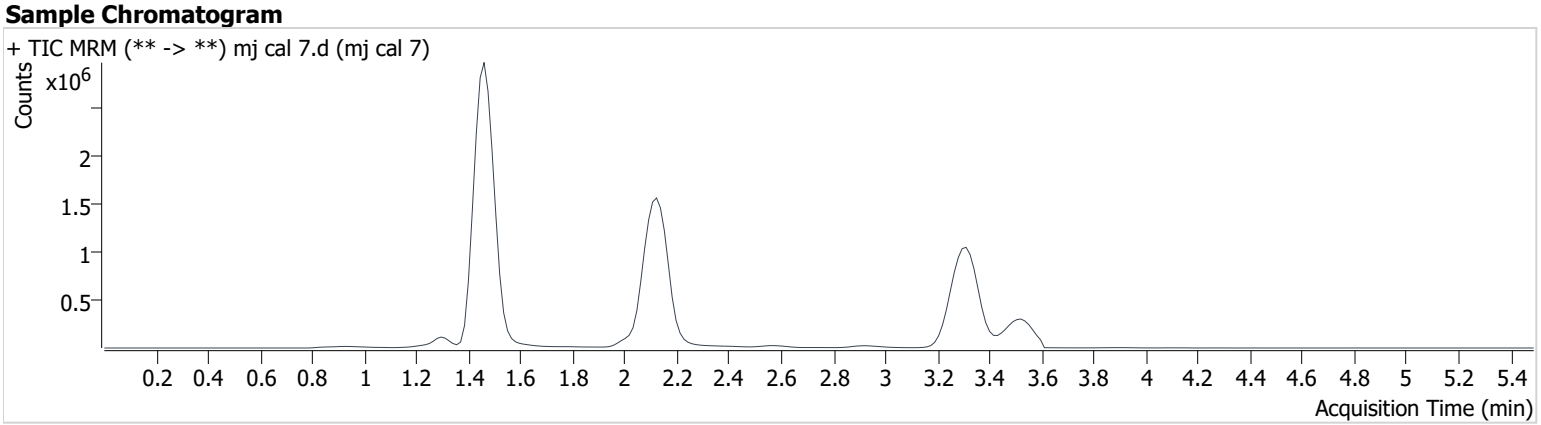
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	3001292	∞	11.4	∞	2060597	49.508	ng/ml
THC-COOH	1.476	1006755	862.7	36.0	1738.5	723585	97.914	ng/ml
THC	3.317	1811336	∞	23.5	∞	1276122	50.070	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:22:45 PM		
Sample Info.			

Sample Chromatogram



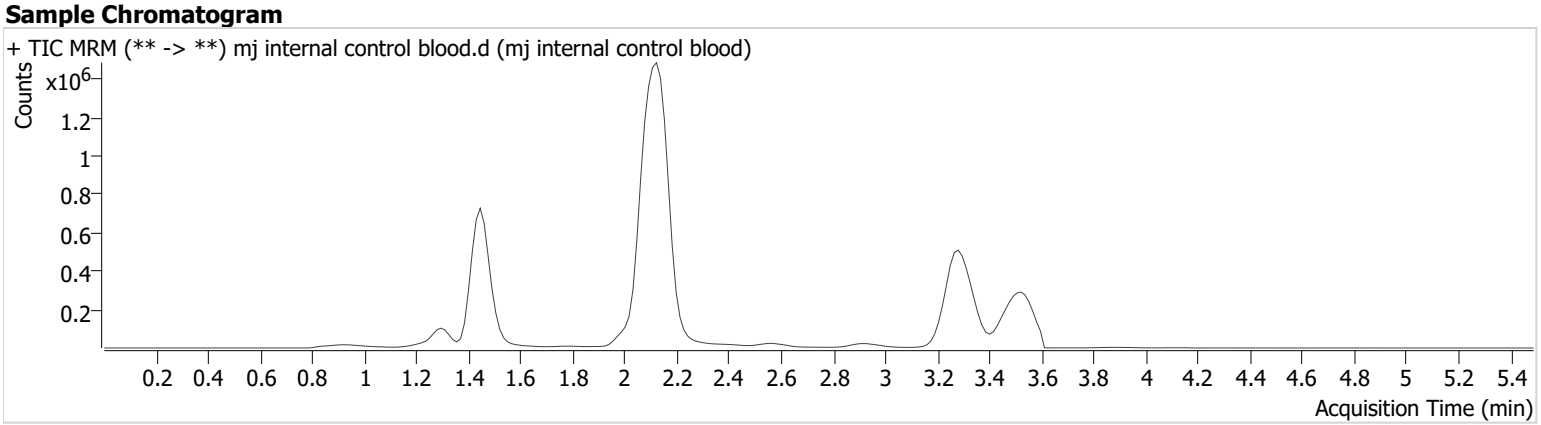
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	5866141	∞	11.9	∞	1983355	100.565	ng/ml
THC-COOH	1.476	2379178	8231.8	37.3	2326665.8	654467	254.690	ng/ml
THC	3.332	3658693	∞	23.8	∞	1260473	102.049	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\042921\QuantResults\cannq.batch.bin
Calibration Last Update 4/29/2021 9:26:33 PM

Instrument	69679	**Data File**	mj internal control blood.d
Type	QC	**Sample**	mj internal control blood
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/29/2021 7:29:29 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	331726	∞	9.9	∞	2160567	5.192	ng/ml
THC-COOH	1.476	153553	1605.7	34.6	532.1	793028	14.235	ng/ml
THC	3.317	160158	∞	25.8	∞	1412582	4.301	ng/ml